学校编码： 10384 分类号_____密级_____
学号：21620100153876 UDC____

厦门大学

博 士 学 位 论 文

菲和芘的心脏发育毒性及其机制的研究

Effects of phenanthrene and pyrene on cardiac development of embryonic zebrafish (*Danio rerio*): the mechanism involved

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论文提交日期： 2013 年 5 月
论文答辩时间： 2013 年 6 月
学位授予日期：

答辩委员会主席：_____
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2013 年 5 月

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摘要

多环芳烃 (Polycyclic aromatic hydrocarbons, PAHs) 是一类广泛分布于环境中的持久性有机污染物 (persistent organic pollutants，POPs)。 众多研究表明，PAHs 不仅具有较强的致癌、致畸和致突变性，还具有胚胎发育毒性，尤其是心脏发育毒性。但到目前为止， PAHs 心脏毒性的研究多集中于高浓度的急性暴露，而对环境浓度的 PAHs 暴露是否会同样的引发心脏发育毒性，研究还甚少；且关于低环数的 PAHs 的心脏发育毒性机制研究尚不清楚。本研究分别选取 3 环的菲 （phenanthrene， Phe）和 4 环的芘 （pyrene， Pyr），在环境水平（0，0.05，5，50 nM）暴露斑马鱼 (*Danio rerio*) 胚胎，应用生态毒理学和分子生物学技术检测在胚胎发育过程中其对心脏发育的毒性效应，旨在探讨：(1) 环境浓度的 Phe、Pyr 暴露是否同样会引发心脏发育缺陷；(2) 比较 Phe、 Pyr 暴露对鱼类心脏发育毒性效应的差异；(3) 比较分析其可能的作用机制。结果显示：

1）斑马鱼受精卵在环境水平的 Phe 暴露下，在胚胎发育过程中，各处理组心脏均产生一定的畸形，且总的心脏畸形率呈显著性上升，主要畸形特征表现为：心包囊水肿、心房与心室重叠减少、心室扩大等。通过 HE 染色等组织学方法分析显示，Phe 暴露能引起斑马鱼胚胎心室腔变大，心室壁变薄，心室心肌细胞层胶原含量增加等畸形。同时，与心脏形态形成相关的 MMP-9 基因，不论是转录水平、蛋白水平还是蛋白活性均呈显著性上升，且通过使用 MMP-9 抑制剂特异性的抑制 MMP-9 活性，Phe 暴露引发的心脏发育缺陷在很大程度上得到了缓解。另外，心室细胞层中，在心肌纤维化过程中与胶原合成密切相关的调控因子 TGF-β 的表达也呈显著性上升。这些结果表明，Phe 暴露可能通过诱导 MMP-9 表达、增加 MMP-9 活性，促进心肌细胞间质 ECM 降解、导致腔室壁变薄；Phe 暴露同时诱发 TGF-β1 调节的胶原合成的增加，最终导致心脏畸形。

2）环境浓度的 Phe 暴露不仅对心脏形态发育产生不良影响，同时也导致心脏机能障碍。Phe 暴露 72h，各处理组心跳速度呈显著性上升，且表现出严重的不规律性, 舒张/收缩末期容积（EDV、ESV）上升，每搏输出量（SV）下降，心输出量（CO）减少等。通过体外暴露大鼠心肌细胞研究发现，Phe 暴露导致

细胞胞浆内 Ca^{2+} 滞留严重，Ca^{2+}浓度呈显著性上升，而肌浆网内储存的 Ca^{2+} 则明显减少；肌浆网钙泵 ATP 酶 SERCA2a 基因转录水平、蛋白表达及其活性均显著性下降；另外，SERCA2a 的一个重要调节因子 Tbx5 的表达也显著性下降。这些结果表明，Phe 可能通过抑制 Tbx5 表达，使得 SERCA2a 表达降低，心肌细胞肌浆网对胞浆中 Ca^{2+} 摄取减少，胞浆中 Ca^{2+} 滞留而扰乱了心肌细胞内钙离子平衡，最终导致收缩、舒张紊乱并引发心率不齐。

3）环境浓度的 Pyr 暴露引发与 Phe 类似的心脏发育毒性，如：心包囊水肿，心脏非正常环化，心室壁变薄，心室细胞层胶原含量增加，心跳加快等。但是 Pyr 暴露 72h，EDV、ESV 均表现出不同程度的下降趋势，且都在 50 nM 呈显著性下降，SV 在 50 nM 处理组显著性减小，CO 则没有发生明显的变化。然而，在作用机制上 Pyr 和 Phe 却不尽相同，Pyr 暴露并不会引起斑马鱼胚胎 MMP-9 基因表达的变化，Bmp2b，Nkx2.5 基因的表达却都呈显著性下降。因此，Pyr 暴露可能是通过影响 Bmp2b 的表达调节 Nkx2.5，从而影响心脏心肌细胞的分化，进而导致心脏发育缺陷。

综上所述，本研究表明环境浓度 Phe 和 Pyr 暴露均能引发类似的心脏发育毒性，但在作用机制上两者却不尽相同。Phe 主要是通过 MMP-9 介导的相关途径诱发心脏发育缺陷和通过扰乱心肌细胞 Ca^{2+} 平衡引发心脏功能障碍，而 Pyr 可能是通过 Bmp2b-Nkx2.5 途径而发生作用的。

关键词：多环芳烃　斑马鱼 胚胎发育　心脏　钙离子 机制

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are a kind of the most abundant and ubiquitous persistent organic pollutants (POPs) in environment. It is widely accepted that PAHs not only have carcinogenic, teratogenic, and mutagenic toxicity, but also developmental toxicity, especially the cardiotoxic effects. However, most of the studies concerning PAHs focus on high dose exposure, and the effects of exposure embryos to environmentally relevant levels of PAHs are still limited. In the present study, zebrafish (*Danio rerio*) embryos were exposed to Phe (phenanthrene) and Pyr (pyrene) at doses of 0, 0.05, 0.5, 5 and 50 nM, and the aims of this thesis were: (1) whether exposure to environmentally relevant levels of Phe and Pyr could cause cardiac defects; (2) What are the differences of cardiac toxicity between Phe and Pyr, and (3) What are the mechanism(s) underlying. The results were as follows:

1) Exposure to environmentally relevant levels of Phe (0.05-50 nmol/L) induced a series of cardiac defects, which were characterized by abnormally looped and enlarged hearts, dilated and thinner ventricular walls, and increased interstitial fibrosis, and the frequency of these abnormalities increased in a dose-dependent manner. Meanwhile, the mRNA and protein expression levels of matrix metalloproteinase-9 (MMP-9), as well as the MMP-9 activity, were induced. Moreover, during co-treatment of the zebrafish embryos with MMP-9 inhibitor, the cardiac defects caused by Phe were attenuated. In addition, Phe exposure led to an up-regulation of transforming growth factor β (TGF-β), which plays a crucial role in mediating cardiac fibrosis. Taken together, our data indicated that the exposure to Phe of zebrafish embryos disrupted normal cardiac development, and that the cardiac defects induced by Phe were mediated by the MMP-9, while TGF-β was also involved in these cardiac defects.

2) Phe exposure also affected cardiac function of zebrafish larvae. By 72hpf (hours

post-fertilization), the heart rate was dramatically increased, which togethered with siginificant arrhythmia, both the EDV (End-diastolic volume) and ESV(End-systolic volume) of the ventricle were also increased, and the SV (stroke volume) and CO (cardiac output) were decreased. Meanwhile, a disordered calcium (Ca^{2+}) handling characterized by impaired sarcoplasmic reticulum (SR) Ca^{2+} uptaking and obvious Ca^{2+} accumulation in cytoplasm was observed in rat embryonic cardiac myoblasts (H9C2) exposed to Phe. The mRNA level as well as protein expression of SERCA2a Ca^{2+} pump in zebrafish hearts or H9C2 cells were significantly decreased by Phe exposure. The activity of Ca^{2+}-ATPase in H9C2 cells was inhibited by Phe. Both the mRNA and protein level of TBX5, a direct regulator of SERCA2a, were significantly decreased by Phe exposure. These results suggested that the exposure to Phe could lead to irregular cardiac rhythm in zebrafish embryos via perturbing calcium handling pathway.

3) Pyr exposure also caused cardiac defects in zebrafish larvae. By 72 hpf, Pyr-treated embryos showed dose-dependent heart abnormalities, such as pericardial edema, cardiac looping defects and increased interstitial fibrosis. They also showed an abnormal contraction including increase of heart beat and decreased EDV, ESV and SV. However, the expression of MMP-9 and the activities were not obviously changed in response to Pyr exposure. The homeodomain transcription factor Nkx2.5 and the bone morphogenetic protein 2b (Bmp2b) were down-regulated. Taken together, these data indicated that embryonic exposure to low-level environmental Pyr disrupt normal cardiac development and alter expression of defective cardiac differentiation related genes.

In summary, the most important finding in our study was that embryonic exposure to both Phe and Pyr could cause some similar cardiac defects. However, the underlying mechanisms of cardiac toxicity were different: The cardiac defects induced by Phe were mediated by the MMP-9, and Phe also could lead to irregular cardiac rhythm via perturbing calcium handling pathway. And Pyr exposure might be through a Bmp2b-Nkx2.5 mediated pathway.

Keywords: PAHs, zebrafish, embryo development, heart, calcium, mechanism

第一章 前言

1.1 多环芳烃污染及研究现状

1.1.1 多环芳烃的理化性质

多环芳烃 (Polycyclic aromatic hydrocarbons, PAHs) 是一类由 2 个或 2 个以上苯环以线性排列、弯接或簇聚的方式连接而成的碳氢化合物，主要来源于煤、碳、石油、木材、有机高分子化合物等有机物的不完全燃烧或燃解过程，是重要的环境和食品污染物。迄今已发现有 200 多种 PAHs，图 1-1 中列出的为 16 种常见的 PAHs，其中有相当部分具有致癌性，如苯并(α)芘 [benzo(a)pyrene, B(a)P]，苯并(α)蒽（benzanthracene, BaA）等。PAHs 大部分是无色或淡黄色的结晶，个别具深色，熔点及沸点较高，蒸气压很小，水溶性较差，脂溶性较强，可在生物体内蓄积，能溶于丙酮、苯、二氯甲烷等有机溶剂。

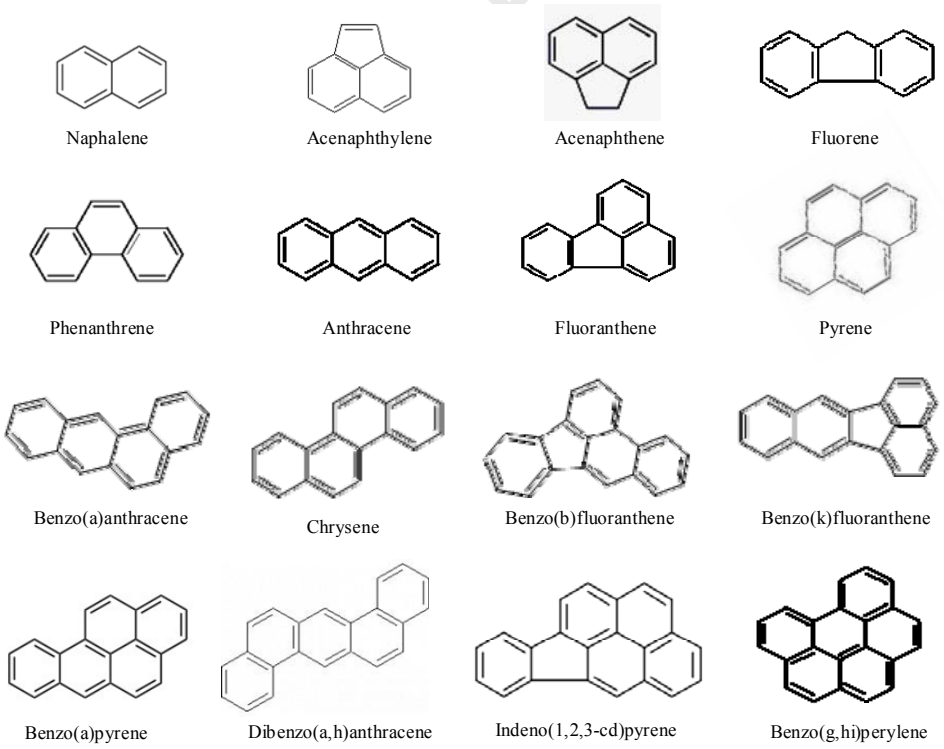

图 1-1 16 种常见的 PAHs

Fig. 1-1 16 kinds of PAHs